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Endocannabinoid System in Neurological Disorders

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Abstract: ***Background:*** Several studies support the evidence that the endocannabinoid system and cannabimimetic drugs might have therapeutic potential in numerous pathologies. These pathologies range from neurological disorders, atherosclerosis, stroke, cancer to obesity/metabolic syndrome and others.

Methods: In this paper we review the endocannabinoid system signaling and its alteration in neurodegenerative disorders like multiple sclerosis, Alzheimer's disease, Parkinson's disease and Huntington's disease and discuss the main findings about the use of cannabinoids in the therapy of these pathologies.

Results: Despite different etiologies, neurodegenerative disorders exhibit similar mechanisms like neuro-inflammation, excitotoxicity, deregulation of intercellular communication, mitochondrial dysfunction and disruption of brain tissue homeostasis. Current treatments ameliorate the symptoms but are not curative. Interfering with the endocannabinoid signaling might be a valid therapeutic option in neuro-degeneration. To this aim, pharmacological intervention to modulate the endocannabinoid system and the use of natural and synthetic cannabimimetic drugs have been assessed. CB1 and CB2 receptor signaling contributes to the control of Ca^{2+} homeostasis, trophic support, mitochondrial activity, and inflammatory conditions.

Conclusion: Several studies and patents suggest that the endocannabinoid system has neuro-protective properties and might be a target in neurodegenerative diseases.

Keywords: Endocannabinoid system, endocannabinoids, cannabinoid receptors, neurological disorders, multiple sclerosis, Alzheimer's disease, Parkinson's disease, Huntington's disease.

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INTRODUCTION

Cannabis is constituted by numerous compounds, but its main component is Δ^9-tetrahydrocannabinol (Δ^9-THC) [1]. CB1 and CB2 receptors are selective cannabinoid receptors identified in neuronal and peripheral cells, respectively. THC mainly stimulates CB1 receptors that are found throughout the brain [2, 3]. After the identification of these receptors, endocannabinoids, anandamide and 2-arachidonoyl glycerol (2-AG) have been detected in mammals and nervous tissues [4, 5], further, their synthetic and degrading enzymes have been also discovered [4, 6-9]. The endocannabinoid system is composed of cannabinoid receptors, endocannabinoids and their synthetic and inactivating enzymes. Extensive research in the last decade has consolidated the view that endocannabinoids function throughout the central nervous system as powerful regulators of synapse by inhibiting transmitter release *via* transient or long-lasting mechanism [10-14]. In particular, the endocannabinoid that is produced by postsynaptic activity, goes backward throughout the synapse, binds the CB1 receptor and inhibits the production of neurotransmitters. Many lines of evidence also suggest a non-retrograde or autocrine signaling of endocannabinoids that modulate neural activity and synapse transmission ~~via~~ transient receptor potential vanilloid ~~receptor~~ type 1 (TRPV1). Furthermore, recent studies suggest a regulation of presynaptic or postsynaptic activity by endocannabinoids *via* astrocytes. Indeed, the endocannabinoid system has been suggested to affect synapse formation and neurogenesis [15]. It is also accepted that by controlling the synaptic strength, endocannabinoids can regulate neural activities like movement control, cognition, feeding and pain. Additionally, a dysregulated endocannabinoid system ~~affects~~ neuropsychiatric disorders like depression and anxiety [16, 17]. Thus, the endocannabinoid system represents an important therapeutic target [18, 19].

In pathologies like multiple sclerosis, Huntington's, Parkinson's and Alzheimer's diseases, and amyotrophic lateral sclerosis, reports suggest symptomatic relief with *cannabis*. These observations suggest that an altered endocannabinoid system might be responsible for several symptoms.

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The finding that the CB1 receptor is highly expressed in areas of the brain like basal ganglia, cortex, cerebellum, hippocampus that affect cognition and motor function, further confirms the role of the endocannabinoid system in a variety of central nervous system disorders like neurodegenerative diseases. Numerous reports suggest the relevant role of the endocannabinoid system in neurological diseases like Alzheimer's disease, and Huntington's disease [20, 21]. Several cannabinoid receptor dependent or independent activities contribute to the pathophysiology of these disorders, like antioxidant function of cannabinoids, stimulation of cytoprotective pathways (protein kinase A (PKA) and B (PKB)), and control of the immune response [22-27].

THE ENDOCANNABINOID SYSTEM: CANNABINOID RECEPTORS

Up to date, CB1 [2] and CB2 [28] receptors have been identified and cloned from mammalian tissues. They are transmembrane ~~receptors coupled to G protein~~ (G_i/G_o). These receptors are membrane embedded and consist of an extracellular N-terminus domain and an intracellular C-terminus domain (Fig. **1**). Glycosylated forms of these receptors are also known [29, 30]. The CB1 receptor is mainly found in the brain [2], while the CB2 receptor has been detected predominantly in peripheral immune cells [28, 31]. The cDNA of the CB1 receptor was isolated from rat brain and its presence was confirmed by transfection of the clone into Chinese hamster ovary (CHO) cell line [2]. Soon after the cloning of the human CB1 receptor [32], the human CB2 receptor cDNA was detected by polymerase chain reaction in differentiated myeloid cells [28]. The presence of the CB2 receptor mRNA was also observed in the spleen and immune cells [33, 34]. CB2 and CB1 receptors have 44% amino acid identity, but differently from the CB1, mouse and human sequences of the CB2 receptor revealed divergent. This suggests the possibility of a certain specificity among species. Griffin *et al.* [35] cloned and expressed the CB2 receptor gene from rat and compared its properties with those from mouse and human: 90% nucleic acid similarity was observed between rat and mouse and 81% between rat and human. Many of the known psychoactive effects of cannabinoids have been attributed to the CB1 receptor, while on the basis of its localization and cannabinoids' properties in immune cells, an immune regulatory role has been ascribed to the CB2 receptor [29, 30].

CB1 RECEPTOR EXPRESSION IN THE CENTRAL NERVOUS SYSTEM

CB1 receptors are G-protein coupled receptor (GPCR) widely found in the brain; even if their expression has also been detected in the periphery. In the central nervous system, high expression of CB1 receptor is observed in basal ganglia nuclei, in prefrontal cortex, in globus pallidus, in substantia nigra while a moderate expression occurs in hippocampus, in cerebellum and neocortex. In the striatum, high levels of the CB1 receptors are found while low mRNA and protein levels occur within the nucleus accumbens [20, 36]. It was suggested that in the nucleus accumbens the CB1 receptor plays a role in behavioral alterations induced by cocaine [37]. The expression profile of the CB1 receptor throughout the brain and main neuronal functions associated with each area of the brain are shown in Fig. (**2**). Cannabinoid-mediated actions like antinociception, catalepsy, hypothermia, hypolocomotion, and

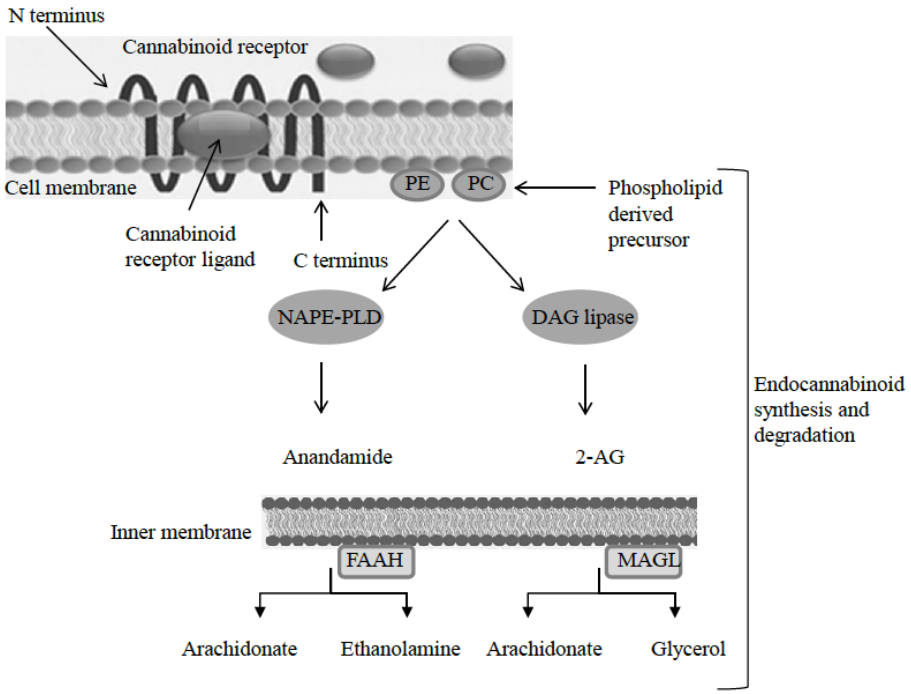

Fig. (1). Scheme of endocannabinoid system. In this figure a schematic representation of the endocannabinoid system is illustrated. On the cell membrane cannabinoid receptors can engage endocannabinoids that are produced on demand by their specific enzymes. The scheme of main endocannabinoids, anandamide and 2-AG synthesis and degradation are also reported. The enzymes NAPE-PLD and ~~DAG lipase~~ synthesize anandamide and 2-AG, respectively. The enzyme FAAH hydrolyzes anandamide in arachidonate and ethanolamine while MAGL degrades 2-AG in arachidonate and glycerol.

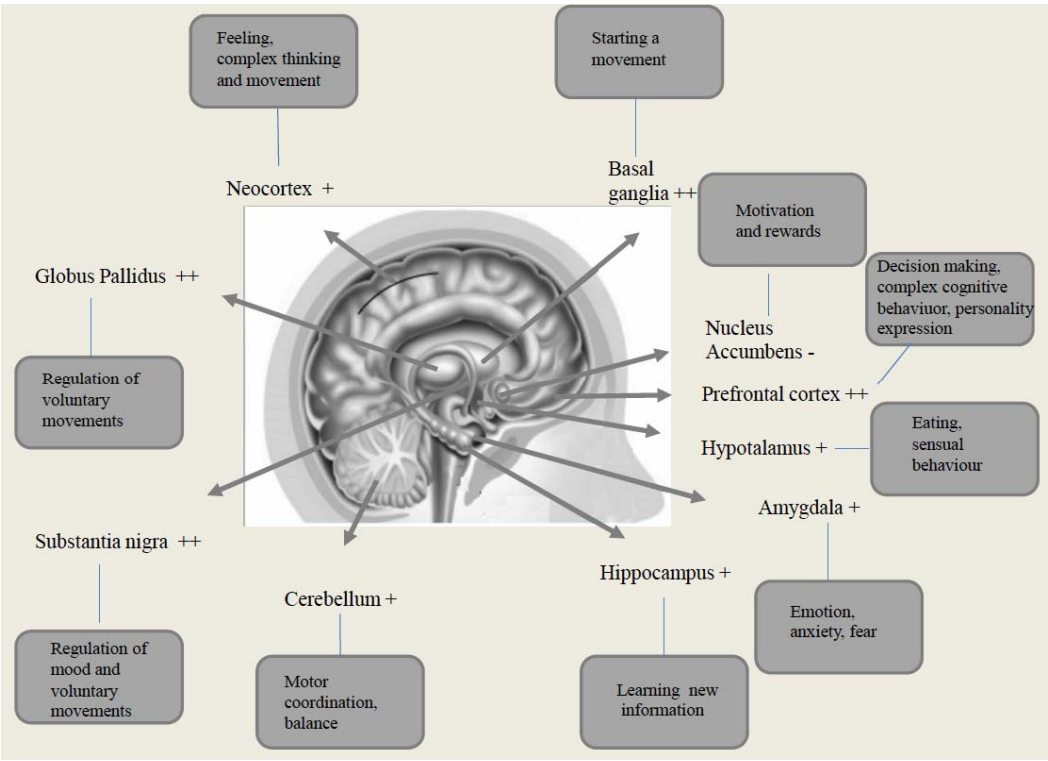

Fig. (2). Localization of CB1 receptors in brain regions. Main areas of the brain are indicated in the figure along with their particular cerebral functions. The localization of the CB1 receptor is reported for each cerebral area. CB1 receptor expression can be very high (++) high (+) and low (-).

memory alteration are associated with the CB1 receptor expression [3]. At the subcellular levels, CB1 receptor has been detected in pre-synaptic terminals, and has been observed at higher levels on GABAergic than glutamatergic neurons in several areas [38-40]. In the periphery, CB1 receptor is found at lower levels, its expression has been revealed in several circulating immune cells [41], and in resident microglia in rat [42]. In microglia, the activation of the CB1 receptor has been demonstrated to block nitric oxide release, suggesting that CB1 receptor may exert anti-inflammatory action [43]. There is evidence that modifications of the expression of the CB1 receptor may occur with stimulation of immune cells; however, conflicting results did not clarify whether the CB1 receptor is up- or down-regulated in these stimulated cells [44]. The expression of CB1 receptor has been detected in astrocytes [45, 46], also after injury [47]; however, it is controversial if it is expressed *in situ* by normal astrocytes. Some reports described no overlap between astrocytic and CB1 receptor immunostaining [48-50], however, more accurate studies are needed to establish that the CB1 receptor expression on perivascular glia observed by other authors was indeed on astrocytes [51-53]. A further investigation in hippocampal slices demonstrated that the response to cannabinoids by astrocytes was inhibited by a CB1 receptor -selective antagonist [54].

CB2 RECEPTOR EXPRESSION IN THE CENTRAL NERVOUS SYSTEM

The question dealing with the expression of CB2 receptor on neurons is still controversial, it was previously suggested that CB2 receptor was absent from the brain. Northern blotting and hybridization assays established a lack of Cnr2 message in the brain in agreement with other reports suggesting no neuronal expression of the CB2 receptors under normal circumstances [28]. However, by real-time PCR low levels of Cnr2 message were detected in the brain [55] in resident microglial cells and not neuronal cells [55]. Notably, other reports have described abundant expression on neurons. This apparent discrepancy might be attributed to poorly validated antibodies as suggested by another ~~report~~ showing methodological problems observed using three different commercial CB2 receptor antibodies. In particular, each of the antibodies bound to several areas of the central nervous system, but they were not able to specifically recognize CB2 receptor as assessed by similar staining patterns in CB2 receptor deficient mice [56, 57]. Thus a clarification about CB2 receptor neuronal expression in health or disease requires the development of more specific reagents.

However, numerous reports have detected the CB2 receptor in diseased neuronal cells, in astrocytomas [58, 59], in microglial and astrocyte cells in Alzheimer's disease [60, 61], and in astrocytes, microglia and T cells in multiple sclerosis [62]. These studies provide clear demonstration of CB2 receptor up-regulation in response to immune cell activation or inflammatory cues. However, a specific marker to discriminate between microglial cells and macrophages still remains to be determined, making hard to establish if up-regulation of the CB2 receptor is ascribable to stimulated resident microglial cells or peripheral macrophages penetrating the brain. Recent studies showed CB2 receptor expression in normal brain; in neural progenitors [63], in neuronal subsets in the brainstem [64], in the spinal cord [65], in microglial populations [66-68], in cerebellar granule layer [69],

but not in normal astrocytes [47, 62, 68, 70]. Several other reports focused on the CB2 receptor immunoreactivity in neuronal populations provided controversial results, thus further investigations are required to clarify this issue [71, 72].

CANNABINOID RECEPTOR SIGNALING

The signaling of the CB1 and CB2 receptors is remarkably complex. These receptors couple mainly to the $G_{i/o}$ subtypes of G protein. Cannabinoids coupling to adenylate cyclase via $G_{i/o}$ usually inhibit cyclase activity through the release of $G_{i\alpha}$ subunits, however, they are also able to activate isoforms 2, 4, or 7 of adenylate cyclase *via* the release of βγ isoforms [73]. Also the simultaneous stimulation of CB1 receptor and dopamine receptors (D2) results in the activation of adenylate cyclase [20], probably as consequence of heterodimerization of these receptors [74]. Although direct proofs that CB1 receptors couple to $G_{q/11}$ are still missing [75], the cannabinoid agonist WIN 55,212-2 demonstrated to enhance intracellular calcium in hippocampus and in human embryonic kidney via $G_{q/11}$ proteins [76]. Indeed, cannabinoid drugs can also block calcium channels [77, 78] and activate potassium channels [79] via βγ subunits of G proteins [80]. Cannabinoids can also stimulate mitogen-activated protein kinases, p38 [81, 82], p44/42 MAP kinase [83, 84], JUN-terminal kinase [85, 86] and activate the phosphatidylinositol-3-kinase pathway [87]. Related effects can be G protein dependent [84, 88] or independent and mediated by other adaptor molecules [89]. Cannabinoids can activate another G protein- independent pathway that involves G protein-coupled receptor kinase-3 and β-arrestin-2, needed for desensitization, but not for internalization of CB1 receptors [90]. Cannabinoids control the activity of phosphatase, [91] and the activation of mitogen-activated protein kinase phosphatase 1 that exerts a relevant function in the anti-inflammatory effects of anandamide [92]. Cannabinoid receptor agonists belonging to different structural classes can induce different signaling cascades that influence the efficiency of these agonists. CB1 receptors can couple and stimulate G_i and G_o, while CB2 receptors only activate G_o. Of note, the efficiency of a given agonist differs if CB1 receptors couple to G_i or G_o, thus suggesting agonist-specific G protein signaling [93, 94]. More striking is the result demonstrating agonist-specific stimulation of different $G_{i\alpha}$ subunits [95]. The selectivity of this agonist in G protein activation might be at least in part due to the presence of different binding sites for different classes of agonists, as described by mutagenesis and molecular modeling reports [96]. In the brain, a relevant characteristic of cannabinoid signaling pathways is the absence of correlation between the density of CB1 receptor in a given area of the brain and the efficacy of receptor coupling, as demonstrated by GTPγS binding assays [97]. This may clarify why functional responses can occur in regions of the brain presenting very sparse CB1 receptor expression, like the brainstem [98] or the hypothalamus [99]. It was observed that in heterozygote mice, the reduction of CB1 receptor density was balanced by an enhanced receptor/G protein coupling efficacy observed for some agonists [100]. Other findings suggest that cannabinoid psychomotor effects can be ascribed to signaling cascades in striatal projection neuronal cells that involve kinase A-mediated phosphorylation of DARPP-32, through regulation of adenosine A2A and dopamine D2 transmission [101]. These results indicate an exclusive form of amplification of CB1 receptor signaling, since DARPP-32 phosphorylation enhances downstream events by block of protein phosphatase-1 [102].

THE ENDOCANNABINOID SYSTEM: ENDOCANNABINOIDS

The discovery of endogenous ligands (endocannabinoids) able to bind to cannabinoid receptors led to the identification of the endocannabinoid system [4, 5]. Endocannabinoids are fatty acid compounds of which anandamide (arachidonoyl ethanolamide) and 2-AG are the best known, their chemical structure is reported in Fig. (**3**). Other polyunsaturated ethanolamines that bind to the cannabinoid receptors have been identified, among these ligands virodhamine, 2-arachidonyl glycerylether (noladine ether) and N-arachidonoyl dopamine have been described [103-105]. In particular, noladine ether is interesting because it was demonstrated to be a full agonist at CB2 receptor [106]. The first endogenous ligand described was anandamide [4, 107], a partial CB1 receptor agonist [93, 108-110] and a weak CB2 receptor partial agonist [111, 112]. 2-AG identified in canine gut and brain [5, 7] is a full CB2 receptor agonist [111] and lacks both receptor affinity and efficacy in humans [110]. Furthermore, 2-AG is the main endocannabinoid for CB2 receptor [113]. It is believed that endocannabinoids can also activate other non - cannabinoid receptors: anandamide binds to the vanilloid receptor type 1 [114-116] and both anandamide and 2-AG activate peroxisome proliferator-activated receptors (PPAR) [117-119]. In addition, anandamide and 2-AG have been involved in several pathological mechanisms by binding the orphan receptor GPR55, which may act as a novel cannabinoid receptor and may represent a novel target for the treatment of pain and inflammatory conditions [119-121].

THE ENDOCANNABINOID SYSTEM: ENDOCANNABINOID SYNTHESIS AND DEGRADATION

The endocannabinoid synthesis and degradation are regulated by complex enzymatic cascades [122]. Five hydrolytic enzymes are involved: two of them, NAPE-selective phospholipase D (NAPE-PLD) and fatty acid amide hydrolase (FAAH) catalyze the production and inactivation of anandamide, respectively; the other three sn-1-selective diacylglycerol lipases (DAGL) alpha and beta, and monoacylglycerol lipase (MAGL) catalyze 2-AG synthesis and degradation. After their production, endocannabinoids can bind to cannabinoid receptors, either after release into the extracellular space or moving within the cellular membrane. The endocannabinoid membrane transporter seems to favor both the endocannabinoid release and re-uptake. The enzyme FAAH degrades anandamide in arachidonate and ethanolamine and MAGL hydrolyzes 2-AG in arachidonate and glycerol, a representative scheme of endocannabinoid production and degradation is reported in Fig. (**1**). The enzymes of these pathways might be potential pharmacological targets in numerous diseases in which these enzymes are abnormally functioning. CB1 and CB2 receptors represent the main targets of anandamide and 2-AG which bind them with differ-

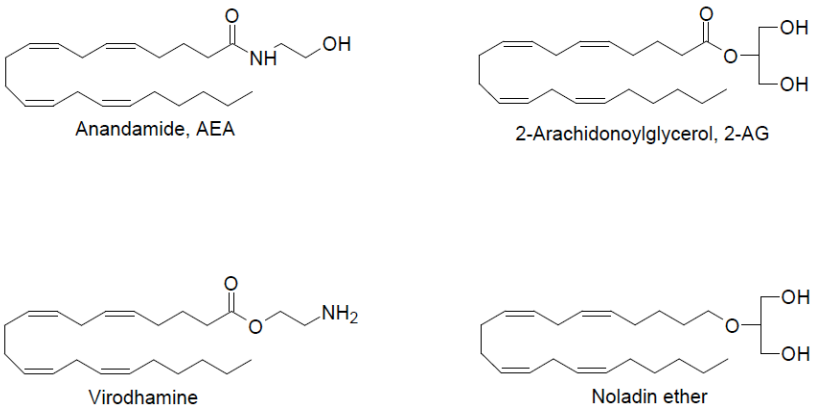

Fig. (3). Chemical structure of endocannabinoids. Main endocannabinoid structures of anandamide, 2-AG, noladine ether and virodhamine are reported in the figure.

ent affinity [123]. Anandamide is CB1 and CB2 receptor partial agonist while 2-AG is CB1 and CB2 receptor full agonist; however, apart from their binding to cannabinoid receptors, endocannabinoids may activate other receptors. Anandamide may bind to TRPV1 [114] and PPAR [124, 125] and is involved in many pathological processes by activation of the orphan receptor GPR55 [119] that might exert a physiological function in lipid or vascular biology [120]. Unlike classical neurotransmitters and neuropeptides, endocannabinoids are produced "on demand" in response to physiological and pathological stimulations [126] and are not stored in intracellular compartments. The endocannabinoid system is highly conserved among species and can modulate proteins and nuclear factors implicated in proliferative responses, cell differentiation and survival [127, 128]. A transient enhancement might be an adaptive reaction to re-establish cell homeostasis when this is altered. For example, high anandamide levels were observed in umbilical vein, placenta and plasma from maternal circulation [129]. Indeed, the alteration of the endocannabinoid system in chronic pathologies seems to favor the progress of neurodegenerative diseases [130]. Enhanced anandamide and 2-AG concentrations have been also detected in several tumors with respect to healthy tissues [131]. Concerning neuronal cells, oligodendrocytes [132], astrocytes, and microglia [133] represent the sites of the synthetic machinery for endocannabinoids. Emerging data show that glia is involved in endocannabinoid signaling [134]. Likewise, cultured microglial cells and astrocytes can produce anandamide or 2-AG [135]. However, it is unclear if endocannabinoids released by glial cells can regulate synaptic transmission. Some findings suggest ~~a role for endocannabinoid in signaling~~ to astrocytes and ~~their ability to~~ regulate synaptic function.

ENDOCANNABINOID AND OTHER NEURONAL SIGNALING SYSTEM INTERACTION

Efficient communication among neurons is necessary for the normal function~~ing~~ of the brain. A main mechanism of neuronal cell communication involves the release of chemical messengers, known as neurotransmitters that on the target cell, bind to specialized receptors, modifying their activity. Pre-synaptic cells synthesize and package these neurotransmitters in vesicles localized on the axons. Neuronal activation determines the release of neurotransmitters from the axonal terminals onto dendrites that are projections in adjacent neurons. Dendrites contain dendritic spines, small protrusions that meet axonal terminals at synapses, specialized points of contact that mediate information exchange between neurons. Neurotransmitter receptors are expressed in target cell dendrites and in axonal terminals and regulate neurotransmitter release. Re-uptake into the axonal terminal or enzymatic breakdown reduce synaptic level of the neurotransmitter, terminating its action. Differently, the endocannabinoid system communicates its messages by a "backward" manner. When the post-synaptic neuron is activated, endocannabinoids (chemical messengers of the endocannabinoid system) are produced "on demand" from lipid precursors (fat cells) that are already present in the neuron. Then, endocannabinoids are released and travel backward to the pre-synaptic neuron, where they bind to cannabinoid receptors. The endocannabinoid system has multiple interactions with other neuro-regulatory systems. In addition to the control of classical neurotransmitter release like GABA and glutamate, CB1 receptor can also modulate the release of other neurotransmitters like serotonin, dopamine, acetylcholine, norepinephrine, cholecystokinin and opioids [13, 136, 137] (a schematic representation is given in Fig. **4A**). A lot of these neurotransmitters couple to endocannabinoid production by stimulating their respective $G_{q/11}$ protein-coupled receptors [14]. ~~In addition,~~ G protein signaling regulators have been demonstrated to ~~regulate~~ $G_{q/11}$ coupled receptors and endocannabinoid mobilization [138]. At the synapse, CB1 receptors functionally interact with other receptors. For example, D2-like receptors co-localize with CB1 receptor in the prefrontal cortex to likely favor CB1 receptor -mediated inhibition of transmitter release [139]. This is probably caused by decreased PKA activity, consistently with similar findings in the ventral tegmental region [140]. Furthermore, it was suggested that brain-derived neurotrophic factor (BDNF) affects CB1 receptor signaling, disrupting endocannabinoid-mediated inhibition of neuromodulator production in visual cortical slices from young mice [141]. BDNF inhibitory effect of CB1 receptor function occurs ~~via~~ a mechanism that requires cholesterol metabolism and perturbed function of membrane lipid raft [142]. In the zone of Schaffer collaterals, a co-localization of adenosine A1 receptors (A1Rs) with CB1 receptor can be observed. Tonic stimula-

tion of A1Rs can decrease the efficiency of CB1 receptor - mediated block of glutamate production [143]. Indeed, in the hippocampus, at inhibitory terminals, GluK1-containing kainate receptor stimulation seems to favor CB1 receptor signaling [144] by a mechanism that is not clear yet. Additional data show that CB1 receptor can couple with other GPCRs to form heteromeric complexes. These coupling interactions have been found for CB1 and D2, CB1 and A2A, CB1 and opioid, CB1 and orexin-1 receptors [145-147] (Fig. **4B**). Of note, also higher order heteromeric aggregates composed of CB1, A2A and D2 receptors have been detected [148].

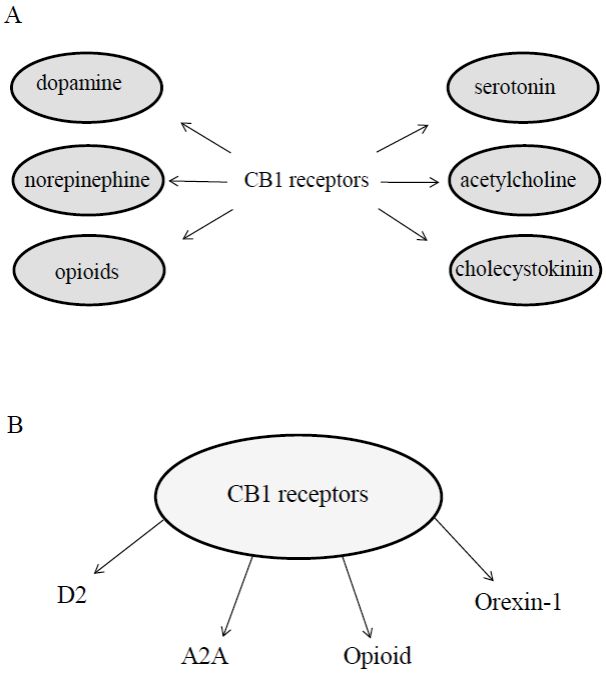

Fig. (4). Interaction of CB1 receptor with other neuronal signals. CB1 receptor controls the release of other neurotransmitters like acetylcholine, serotonin, dopamine, norepinephrine, cholecystokinin and opioids. Lots of these neurotrasmitters couple to endocannabinoids production by stimulating their $G_{q/11}$ protein-coupled receptors (**A**). Interactions between CB1 receptor and other receptors have been described. These interactions have been observed for CB1-orexin-1, CB1-A2A, CB1-opioid and CB1-D2 receptor pairs (**B**).

Such interactions can modify the downstream of G-proteins enrolled during receptor stimulation. Additional studies are needed to establish how these heteromeric complexes can influence physiological conditions in the central nervous system and also at the synapse. Furthermore, the endocannabinoid system undergoes plastic modifications. Plasticity of the endocannabinoid system might be due to changes of its constituents like, CB1 receptor amount/function or endocannabinoid synthesis/inactivation. These modifications observed both *in vitro* and *in vivo*, can be elicited by various natural and experimental settings, for example neural function and agonist-induced CB1 receptor stimulation. Of clinical relevance, the alteration of endocannabinoid signaling can drastically influence synaptic physiology and brain activity and is often associated with brain diseases.

ENDOCANNABINOID SYSTEM AND NEURODEGENERATION

The emerging role of the endocannabinoid system in numerous central nervous system diseases cannot be surprising given the high expression level of the CB1 receptor in the brain. The high amount of CB1 receptors in the cortex, hippocampus, and basal ganglia cerebellum focused the attention to pathologies involving movement, anxiety, mood disorders and diseases associated with perturbed brain reward mechanisms. The classical effects of *cannabis* on behavior provided clues about potential therapeutic targets, like regulation of appetite or pain. In the brain, the endocannabinoid system has been shown to exert neuro-modulatory activity mediating the effects of THC, the psychoactive component of *cannabis*. Two principal pathways by which endocannabinoids may influence neurodegenerative disorder progression have been suggested: neuro-modulation and immuno-modulation. Neuro-modulatory effects of endocannabinoids and the signal transduction of cannabinoid receptors have been well characterized in numerous studies [149-152]. Briefly, it has been determined that endocannabinoids [153] act as retrograde CB1 receptor agonists in the pre-synaptic terminals and block inhibitory or excitatory neuromodulator release by pre-synaptic neuron [154, 155]. Despite evidence supporting that anandamide binds to cerebral CB1 receptors, some of the known cannabimimetic effects of anandamide can be due to its full agonist action on the TRPV1, of which capsaicin, is considered the main exogenous ligand. The endovanilloid function of anandamide may influence numerous physiological cerebral activities as TRPV1 receptors are found both in the central nervous system and in the periphery [79]. Studies suggest that anandamide and 2-AG have specific pharmacological properties, are engaged in different forms of synaptic plasticity and involved in different behavioral functions [156], such as antinociception, learning, memory, reward, addiction and anxiety. Furthermore, endocannabinoids, apart from their key function in the regulatory activity of neurons, exert a crucial role in the function of peripheral and brain immune cells. CB2 receptors are found on several resident and circulating immune cells; in particular, upon immune cell activation by CB2 receptor agonists, pro-inflammatory effects can be elicited. These effects include the block of i) inflammatory mediator release, such as TNF-α, interleukin-2 and nitric oxide, ii) stimulation of processes mediated by immune cells and iii) proliferative response and chemotaxis [27, 157-160].

On the other hand, anecdotal and experimental evidence reported symptomatic relief by *cannabis* in several neurological disorders like multiple sclerosis, amyotrophic lateral sclerosis, Parkinson's, Huntington's, and Alzheimer's diseases. These findings suggest that the symptomatology of these diseases might be ascribed to hypofunction or dysregulation of the endocannabinoid system [17, 161]. The pathophysiology can involve both cannabinoid receptor-mediated and not mediated mechanisms. These mechanisms include antioxidant properties of cannabinoids, induction of cyto-protective pathways and regulation of the immune response ~~via~~ CB1 and CB2 receptors [23, 162-166]. Thus, the modulation of the endocannabinoid system might represent a therapeutic target, indeed, its pharmacological manipulation might be useful to repair injured tissues in neurodegenerative diseases [167-169].

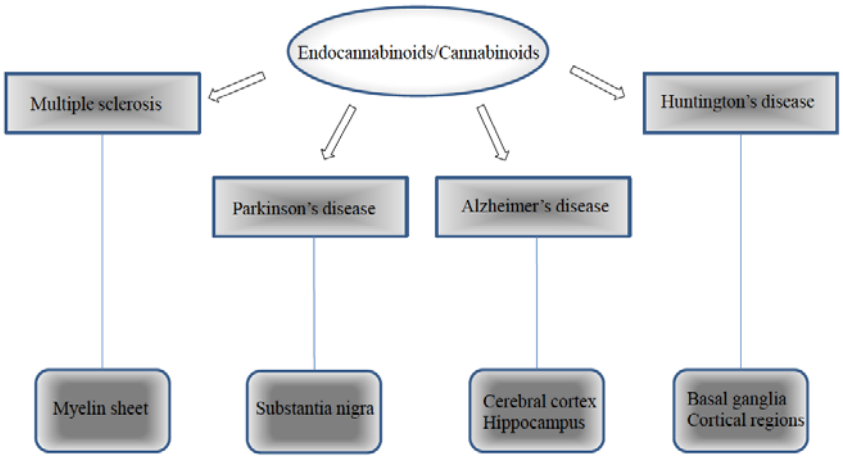

Fig. (5). Neurological diseases as targets of endocannabinoid/cannabinoids. Multiple evidence suggest the involvement of endocannabinoids in the control of motor activity and in movement disorders associated with neurological diseases like Alzheimer's, Huntington's, Parkinson's diseases and multiple sclerosis. The area of the brain region associated with these pathologies is indicated in the figure. Evidence also suggest the benefits derived by the use of cannabinoid-based drugs in main neurological diseases.

However, the use of cannabinoids as therapeutics is still a controversial issue, although they demonstrated to successfully regulate sleep, pain, appetite and some psychotic behaviors. In this context, a recent patent about the use of cannabinoid in neuropathic pain has been published [170]. This invention states the use of phytocannabinoids isolated from the plant to treat allodynic pain. In addition, to further support the antioxidant and neuroprotective properties of cannabinoids, another patent "Cannabinoids as antioxidants and neuroprotectants" [171] shows the applicability of cannabinoids in limiting neuronal damage after ischemia, or in the therapy of neurodegenerative pathologies like Alzheimer's disease, Parkinson's disease and HIV dementia. Another invention [172] defines the use of cannabinoid – containing plant extracts to prevent or treat neural degeneration. The authors support the neuro-protective effects of cannabinoids identified in the plant extracts in pathologies like Alzheimer's, Huntington's, Parkinson's disease, amyotrophic lateral sclerosis, frontotemporal, Lewy body, vascular and HIV dementia, prion disease, progressive supranuclear palsy, normal pressure hydrocephalus, traumatic spinal cord injury, Down's syndrome, alcohol induced neurotoxicity, and epilepsy. A further patent [173] also suggests cannabinoid application in the prevention or treatment of neurodegenerative diseases. Cannabinoids described in this study are cannabichromene (CBC), cannabidivarin (CBDV) and cannabidivarin acid (CBDVA). Some of these compounds decreased the Aβ-dependent glial cell proliferation and activation; or reduced the neurotoxicity induced by Aβ treatment and the transcription of pro-inflammatory proteins. These cannabinoids also decreased the amount of nitrite a key parameter in the study of many neurological diseases.

Their efficacy in neurological diseases is also reviewed in the following sections, focusing on multiple sclerosis, amyotrophic lateral sclerosis, Huntington's disease, Parkinson's disease, and Alzheimer's disease.

ENDOCANNABINOID SYSTEM AND NEURODEGENERATIVE DISEASES

In the brain multiple reports suggest that endocannabinoid system is involved in the control of motor function and movement disorders. In particular, the CB1 receptor is highly expressed in areas of the brain known to be involved in motor control like basal ganglia, cerebellum and substantia nigra [3, 36, 51, 174-178]. These brain regions contain high amount of endocannabinoids [179, 180]; that along with *cannabis*-derived and synthetic compounds exert potent and inhibitory action on motor function [181-187]. Furthermore, in the basal ganglia, CB1 receptors and endocannabinoid levels are altered in both animal models [188-193] and human conditions of motor disorders [187, 191, 194-196]; indeed, the endocannabinoid system interacts with various neuromodulator signaling pathways in the basal ganglia circuitry [197-204]. We report in the section below evidence of the modulation of the endocannabinoid system in neurodegenerative disorders highlighting the therapeutic potential of cannabinoids in these diseases. In Fig. (**5**) a schematic representation of neurodegenerative diseases target of endocannabinoids/cannabinoids is reported as well as the area of the brain mainly involved in each diseases.

MULTIPLE SCLEROSIS

Multiple sclerosis is a chronic disabling disorder of the brain caused by demyelination of nervous fibers. Women are more frequently affected than men and the disease usually begins in young adulthood. Balance problems, fatigue, muscle weakness, incontinence, tremor, pain and muscle spasticity are main symptoms of the disease. Multiple sclerosis is characterized by at least two different forms; one is governed by acute relapses and one by steady progression. Environmental and genetic factors can synergize to favor disease manifestation and progression. Usually the first stage is characterized by a relapsing – remitting phase (RR-MS); over time, relapses diminish and patients experience a progressive neurological deficit that characterizes the secondary progressive phase. However, in few cases, multiple sclerosis starts with a primary progressive phase (PP-MS) with no acute relapses. Usually, when patients enter the secondary progressive phase, the progression rate of this phase is similar to that observed in RR-MS [205]. Frequently RR-MS phase is characterized by lesions usually observed in regions of white matter, by local

oedema, disruption of the blood–brain barrier, and demyelination, that favor an inflammatory profile. This inflammatory process is less conspicuous in ~~PP-MS~~, [206]. However, in the progressive phase predominant global brain atrophy occurs and correlates with disability [207, 208]. These findings suggest that early stages of the disease are governed by ongoing inflammatory activity responsible for the relapsing – remitting phase, while a different process occurs in the progressive phase that is characterized by a decrease of inflammation and a faster progression of disability. Histological hallmarks of active multiple sclerosis include infiltrations of immune cells, myelin degradation and reactive modifications of microglia and astrocytes [209]. Inflammatory lesions are driven by an autoimmune process that favors primary demyelination associated with a block of normal neuro-transmission [210]. Among the symptoms, at some points up to 90% of patients experience spasticity and spasms that lead to considerable decrease of mobility, and interfere with daily activities. In addition, pain, distension of the urinary bladder, and urinary tract infections influence the development and aggravation of spasticity.

LEVELS OF CANNABINOID SYSTEM COMPONENTS IN MULTIPLE SCLEROSIS

Several studies have shown that the endocannabinoid system is modulated in multiple sclerosis. It was observed that anandamide expression is enhanced in multiple sclerosis lesions [92] and that the CB2 receptor is expressed by glia and also in healthy human brain [67], indeed it is up-regulated by neuro-inflammation [60, 212]. Another immunohistochemical study ~~focused on~~ the presence of cannabinoid receptors in human multiple sclerosis samples revealed high CB2 receptor immunoreactivity in microglial/macrophage cells in white matter regions, within or at the edge of plaques [213]. Other studies confirmed an altered endocannabinoid system in the brains of multiple sclerosis patients with respect to normal subjects. In particular, the expression of CB1 ~~and~~, CB2 receptors and FAAH in brain tissue samples obtained from multiple sclerosis patients was investigated. The authors of this study classified areas of demyelination as inactive, active and chronic plaques. Immunohistochemistry and immunofluorescence techniques examined density and cellular localization of CB1 ~~and~~ CB2 receptors and FAAH. In multiple sclerosis samples, CB1 receptor was found in oligodendrocytes, oligodendrocyte precursor cells, in cortical neurons, in macrophages and infiltrated T-cells. On the other hand, CB2 receptor was observed in T-cells, perivascular and reactive microglia and astrocytes in multiple sclerosis plaques. In particular, active plaques were enriched of CB2 receptor-positive microglial cells that were localized at the plaque periphery. FAAH enzyme was detected in neurons and hypertrophic astrocytes. As observed in other neuroinflammatory conditions, CB1, CB2 receptors and FAAH can be induced in glial cells during multiple sclerosis [214], finding suggesting that the endocannabinoid system has a role in the pathogenesis of this disease. Moreover, in mice with asymptomatic or mild experimental autoimmune encephalomyelitis (EAE), that is a model of multiple sclerosis, anandamide and 2-AG production was normal [215, 216] while it was enhanced in the chronic relapsing mouse model [217]. In addition, anandamide and 2-AG levels and the expression of the CB1 receptor decreased in the brains of EAE rats [218-220]. Further studies have evaluated in patients and in EAE mice amount, binding, metabolism and physiological activities of endocannabinoids, the results obtained showed a drastic rearrangement of the endocannabinoid system. In particular, anandamide was enhanced in the cerebrospinal fluid of patients with relapsing multiple sclerosis and its concentration was higher in peripheral lymphocytes. Enhanced level of anandamide was also observed in the brains of mice with acute EAE, suggesting its anti-excitotoxic effect [221]. Further research focused on the analyses of endocannabinoid levels in the cerebrospinal fluid comparing relapsing remitting and secondary progressive phases. Significantly reduced expression~~s~~ of anandamide, palmotylethanolamide, oleoylethanolamide and 2-AG w~~ere~~ found in the cerebrospinal fluid of patients with respect to controls and lower values were observed in the secondary progressive group. Higher anandamide and palmotylethanolamide levels were detected during a relapse in the cerebrospinal fluid of relapsing remitting patients. Finally, also higher anandamide, 2-AG and oleoylethanolamide levels were observed in relapsing remitting patients with lesions, thus suggesting endocannabinoid potential role in reducing the ongoing neuroinflammatory process [222]. A further study investigated the expression of endocannabinoids in plasma of secondary progressive multiple sclerosis patients; in particular, anandamide, palmotylethanolamide and oleoylethanolamide levels were increased. Primary progressive multiple sclerosis patients had higher plasma levels of anandamide with respect to controls. FAAH mRNA was decreased in secondary progressive phase but it was not altered in the remitting phase or in the blood of patients with primary progressive multiple sclerosis. The CB1 and CB2 receptor mRNA was increased in the ~~PP-MS~~. These findings suggest a dynamic modulation of the endocannabinoid system depending on the phase of the disease [223]. A recent study measured cannabinoid CB1 and CB2 receptor gene expression in B, natural killer and T cells from patients before and after 1 year of interferon beta treatment, and compared these levels to those of healthy controls. The expression of anandamide, 2-AG and the gene of FAAH synthesis were also evaluated in the same cells. Before starting the therapy, multiple sclerosis patients showed significantly increased cannabinoid CB2 receptor expression in B lymphocytes, but not in T cells or natural killer cells. These levels slowly decreased within 6 months to 1 year of interferon beta therapy. CB1 receptor expression was increased in all cell subsets, but was statistically significant in T cells; all levels decreased over time. Before treatment, anandamide but not 2-AG levels were enhanced in all cell subsets; after 1 year of interferon beta treatment, all values decreased to control levels. The expression level of FAAH was unchanged. These results suggest a role of the endocannabinoid system in multiple sclerosis immune responses and that it can be modulated by interferon beta therapy [224].

THERAPY OF MULTIPLE SCLEROSIS

Currently, anti-inflammatory, immuno-suppressive and immune-modulatory agents are used for the treatment of multiple sclerosis, but this therapy is only partially efficient and is often accompanied by side effects not easily tolerated by patients. Physiotherapy, despite being the standard ap-

proach for the relief of spasms [225, 226] has not been adequately investigated and is not sufficient alone and the administration of anti-spastic drugs is required. Tizanidine and baclofen are the most commonly used anti-spastic drugs, clinical trials show comparable efficacy between these two drugs, however, tizanidine seems to be favored for its higher tolerability. Thus, although many studies support tizanidine monotherapy, a clinical case trial suggested the efficacy of a combination therapy of tizanidine and baclofen to control spasticity and to better manage dose-dependent side effects, however more studies are required to confirm these findings [227]. Tolperisone and dantrolene are not often prescribed. Benzodiazepines provide sufficient anti-spastic effects, but since adverse events like sedation and dependence, they represent second-line agents [228, 229]. Gabapentin demonstrated efficiency to treat phasic spasticity [230, 231]. These drugs have often limited efficacy to treat focal spasticity, however botulinum toxin type A was showed able to efficiently reduce muscle tone [232, 233]. Numerous placebo-controlled studies and open-label trials have suggested the efficiency of botulinum toxin to ameliorate spasticity in multiple sclerosis, spinal cord and brain injury, stroke and cerebral palsy. Enhanced muscle tone and frequency of spasms are reduced by intrathecal baclofen [234, 235], however, its efficacy is reduced after long-term treatment. Available treatments present scarce efficacy and so far, no protocol to treat spasticity has been developed. Lack of treatments, induced multiple sclerosis patients to use *cannabis*, since anecdotal evidence suggested its efficacy in controlling main symptoms like tremors, pain, spasticity and bladder dysfunction. Indeed, several studies have suggested that anandamide and 2-AG exhibit a neuromodulatory action [236] on the production, release and effect of neurotransmitters, among these neurotransmitters, γ-aminobutyric acid, glutamate and dopamine seem to be involved in the pathogenesis of EAE [237-239].

Studies in Animal Models

Numerous *in vivo* studies documented the effects of cannabinoids in multiple sclerosis. Endogenous and exogenous cannabinoids, *via* cannabinoid receptors, have been reported to ameliorate spasticity and tremors in mice with chronic relapsing experimental allergic encephalomyelitis (CREAE) [240]. THC and R (+)-WIN552122 administered intravenously, rapidly diminished amplitude and frequency of tremors in mice with multiple sclerosis. These effects were likely mediated by cannabinoid receptors, since both the antagonists of CB1 and CB2 receptors, SR141716A and SR144528 respectively, prevented R (+)-WIN552122 from inhibiting tremors. Indeed, cannabidiol and the *S* (-)-enantiomer of WIN552122, that are weak CB1 and CB2 receptor agonists, had no effect on spasticity. A relevant point to be better addressed is represented by the possible psychotropic adverse effects due to intravenous administration of cannabinoid drugs. A stable analogue of anandamide, methanandamide [241] showed the same potency of R (+)-WIN552122 to inhibit hind limb spasticity in CREAE mice, this finding suggests that endocannabinoid–based drugs might represent an alternative treatment for spasticity exhibiting low physical dependence [242]. The anti-inflammatory mediator palmitoylethanolamide (PEA) [243] is another non-psychoactive endogenous agent that elicits a transient block of spasticity [240], however, its mechanism of action likely cannabinoid receptor independent has not been defined yet [243].

In CREAE mice both SR141716A and with less potency SR144528 enhanced spasticity and tremors of tail and hind limbs [240]. This evidence suggests that anandamide and 2-AG [241] might be synthesized during CREAE, to likely balance the spastic defect. Spinal cords and whole brain of ABH normal mice exhibited similar levels of anandamide, PEA and 2-AG [217]. The levels of anandamide were slightly increased in spastic brains with respect to those of normal brains. Indeed, in spinal cord of spastic mice the levels of anandamide, PEA and 2-AG were increased with respect to healthy mice. These findings suggested a therapeutic potential related to the enhanced levels of anandamide since exogenously administrated or naturally occurring cannabinoids can diminish spasticity. In addition, inhibitors of anandamide degradation might represent a good strategy to enhance endocannabinoid bioavailability. The re-uptake inhibitor AM404 [244] or FAAH inhibitor AM374 [245] injected intravenously could ameliorate spasticity, both drugs enhanced anandamide neuro-modulatory actions [244] and displayed low affinity for cannabinoid receptors [244, 245]. Pre-administration of SR141716A and SR144465 blocked AM374 anti-spastic effect. This finding suggests that the inhibition of spasticity by AM374, that does not bind to cannabinoid receptors [245], might be due to increased endocannabinoid levels and subsequent activation of cannabinoid receptors. AM404 and anandamide are also TRPV1 agonists [246-248], but the role of TRPV1 in the control of spasticity has not been determined. VDM11, that is a selective inhibitor of anandamide transporter, without TRPV1 or cannabinoid receptor agonist activity [249], exhibited inhibition of spasticity. Thus, it can be hypothesized that endocannabinoids control spasticity *via* cannabinoid receptors. TRPV1 agonists decrease bladder hyper-reactivity in multiple sclerosis [250] and show low anti-spastic activity in EAE mice, arvanil a synthetic drug that can bind to CB1 and TRPV1 receptors [251] significantly reduced spasticity. Such effect was maintained in the presence of CB1 and TRPV1 receptor antagonists and in CB1 receptor knockout mice with EAE, suggesting that arvanil anti-spastic effect independent from CB2, CB1 or TRPV1 receptors could occur *via* another mechanism. Furthermore, arvanil inhibited the proliferative response of immune cell decreasing IFN-γ levels without induction of apoptosis. In addition, arvanil ameliorated the symptoms of EAE in mice [252]. Further investigations should clarify the efficacy of synthetic molecules like arvanil in the course of the disease. The manipulation of endocannabinoid system may reduce the unwanted psychoactive effects due to CB1 receptor agonism and may control symptoms in multiple sclerosis [253, 254]. Recently, the CB2 receptors emerged as novel therapeutic target for multiple sclerosis and new ligands of these receptors have been described [255, 256]. In particular, the effects of these new CB2 receptor ligands have been evaluated in lymphocytes isolated from multiple sclerosis patients and for some of them, their propensity to cross the blood brain barrier have been described [257, 258]. A very recent study showed that inhibition of 2-AG hydrolytic enzyme, the alpha/beta-hydrolase domain 6 (ABHD6) increased 2-AG brain levels, ameliorated clinical

signs of EAE, reduced T cells infiltration, microglia activation and the expression of activated leukocyte cell adhesion molecules, and this effect was mediated by the CB2 receptors. These data suggest that block of ABHD6 might represent a potential approach for the treatment of multiple sclerosis and might be investigated also in other neurodegenerative diseases [259]. Further, the CB2 receptors might represent an alternative target in the treatment of multiple sclerosis, indeed, the generation of CB2 receptor knockout mice contributed to understand the role of CB2 receptor in immune cell function and development particularly in multiple sclerosis [260, 252].

Human Clinical Studies

"Cannabinoids in Multiple Sclerosis (CAMS)" study represents the first large scale study performed to assess potential beneficial effects of cannabinoids on the symptoms of multiple sclerosis [261]. This randomized, placebo-controlled trial enrolled 630 patients in a stable phase of the disease and with muscle spasticity. 211 patients were treated with oral *cannabis* extract, 206 with Δ^9-THC and 213 with placebo for 15 weeks. In a first instance, muscle spasticity was measured by Ashworth assessment; however, also safety and disability were analyzed. Amelioration of pain and spasticity was reported in 61%, 60%, and 46% of patients on *cannabis* extracts, Δ^9-THC and placebo, respectively. All patients discontinued the therapy during week 14, and no effect of the treatment on other disabilities was reported from baseline to week 13. However, an improvement in ambulatory patients of walking, muscle spasms, pain and sleep was described. Findings observed were consistent with other studies [262-265] and with those from a crossover study [266] indicating a decrease of spasms and an amelioration of mobility in patients receiving *cannabis* extracts. A follow up of CAMS study was performed to evaluate long term effects and safety of cannabinoids in multiple sclerosis. In this study, 630 participants with stable disease and with spasticity randomly received *cannabis* extract, oral Δ9-THC, or placebo for the 15 weeks of the CAMS study. Afterword, patients continued the treatment for the follow- up study for other 12 months. A small effect was observed on spasticity, Δ^9-THC ameliorated in part disability without safety concerns. Patients experienced improvement of their symptoms. In another clinical trial, Sativex that is a combination of THC and cannabidiol (1:1) [267] was well tolerated in healthy control subjects and in patients. Sativex was efficient to improve intractable pain, rheumatoid arthritis and multiple sclerosis, without intoxication like effects, tolerance or withdrawal syndrome [268]. 92% of patients declared as adverse effects dizziness and nausea [269]. Side effects due to long term treatments are still unclear, thus further investigation to explore this issue is required to understand if beyond improvements of symptoms, cannabinoids may have a therapeutic role in multiple sclerosis.

Parkinson's Disease

The second most common neurodegenerative pathology is Parkinson's disease that affect 1% of population over 60 and 4% of population over 80 years of age [270]. Death of dopaminergic neurons in the substantia nigra is the main cause of Parkinson's disease, which determines not sufficient production and action of dopamine. Furthermore, motor cortex stimulation by basal ganglia is reduced, resulting in tremors, rigidity and slowing of physical movements (bradykinesia) [271]. Genetic mutations, inflammation, oxidative stress and exogenous toxins have been associated with this disease [271]. It is possible to distinguish primary symptoms, such as tremors, muscle rigidity, bradykinesia, and secondary symptoms, such as high levels of cognitive dysfunction and language difficulties. In Parkinson's disease, cell dysfunction and death are due to neuro-inflammation, calcium dysregulation, oxidative stress, mitochondrial dysfunction, protein aggregation, and prion-like processes. Moreover, abnormalities in non-dopaminergic transmission probably contribute to the block of motor activity. There is an increase of the inhibitory GABA-ergic transmission from the striatum to the external area of the globus pallidus, consequently GABAergic input from the external region of the globus pallidus to the subthalamic nucleus is reduced. In turn, the subthalamic nucleus results hyperactive and enhances the activity of the internal globus pallidus and the substantia nigra pars reticulate, that through inhibitory output to motor nuclei outside the basal ganglia, seem to contribute to the abnormal motor inhibition [272, 273]. Deregulated neuronal circuits lead to synchronization of basal ganglia outputs, firing rate and anomalous patterning [272, 273]. Of note, non-dopaminergic pathways may counteract the lack of dopamine and may be the cause of the absence of parkinsonian symptoms that occur only when there is the loss of 80% of striatal dopamine.

LEVELS OF CANNABINOID SYSTEM COMPONENTS IN PARKINSON'S DISEASE

Several studies aimed to describe the levels of the CB1 receptor in parkinsonian tissues: a decreased expression of CB1 receptor mRNA in the globus pallidus, anterior dorsal putamen and caudate nucleus was reported, but, in contrast, other studies indicated increased CB1 receptor levels in putamen and caudate nucleus [191, 274]. In a single study, the levels of endocannabinoids in patients with Parkinson's disease were analyzed: in the cerebrospinal fluid of these patients higher levels of anandamide with respect to age-matched controls were reported. Of note, anandamide levels returned to normal levels in patients on dopamine replacement therapy [275]. Indeed, anandamide has been demonstrated to decrease dopamine production in striatal slice cultures and increase it in nucleus accumbens *in vivo* [276, 277]; moreover, the stimulation of D2 receptors has been observed to enhance the levels of anandamide in the basal ganglia [200, 278]. In the reserpine-treated animal model of Parkinson's disease a sevenfold increase in the levels of 2-AG was detected in the globus pallidus, and this finding has been related to inhibition of locomotion [279]. Furthermore, in an animal model of Parkinson's disease a reduced degradation of endocannabinoids has been observed along with decreased levels of FAAH and anandamide membrane transporter in the striatum [280]. In the brain of patients, the increase of both endocannabinoid tone and CB1 receptor activity has been suggested to occur to normalize striatal activity after dopamine depletion, since increased signaling of the CB1 receptor decreases the production of glutamate and in-

duces the pool of G-proteins usually activated by the dopamine D2 receptor [202, 281]. By contrast, studies aimed to evidence a role of the CB2 receptors in Parkinson's disease suggest that this receptor does not represent a relevant target [282].

THERAPY OF PARKINSON'S DISEASE

Common therapies for Parkinson's disease include oral dopamine replacement by levodopa, a dopamine precursor, monoamine oxidase B inhibitors and anticholinergic drugs [283]. The primary choice of symptomatic treatment for Parkinson's disease is levodopa that is efficient to control symptoms in the short term, however, its chronic use is accompanied by motor complications like response oscillations and dyskinesia, which is known to affects 30–35% of patients after 2 years of levodopa exposure and often patients discontinue the treatment and experience severe disability.

Current research is aimed to develop new non-dopaminergic agents able to attenuate motor deficit and restore dopamine transmission without dyskinesia [284]. Among these agents, cannabinoids have demonstrated neuroprotective ability along with their potential to alleviate symptoms of motor deficit. In preclinical studies, *cannabis*-based drugs showed efficacy to reduce neuroinflammation, excitotoxicity, oxidative stress, and motor complications of Parkinson's disease [285]. Furthermore, the endocannabinoid system is correlated with the dopaminergic system, which controls by a reciprocal regulation the endocannabinoids; in fact, D1/D2-like receptors and CB1 receptors exhibit signaling pathways that interact each other [74, 281, 286] and are both found in striatal neurons [178, 287]. In Parkinson's disease, beneficial effects like reduction of tremors due subthalamic nucleus over activity [199, 288], and delay of nigral degeneration have been attributed to CB1 receptor agonists, and to dopamine agonists that improve motor impairments [199, 289, 290]. The neuro-protective effects of many cannabinoid agonists in Parkinson's disease have been highlighted by preclinical studies: Δ^9-THC and cannabidiol were the first drugs able to attenuate nigro-striatal dopaminergic neuron damage due to neurotoxin 6-hydroxydopamine in rats [282, 291].

Δ^9-THC binds to and activates CB1 and CB2 receptors, whereas cannabidiol has no activity on these receptors, the neuro-protective effects of these compounds likely occur *via* their antioxidant properties that are not mediated by cannabinoid receptors [291]. This was confirmed by studies on other compounds endowed with antioxidant properties that showed higher selectivity for both receptors [282, 292]. Similar results were observed in an invertebrate model of Parkinson's disease [293] and in the model of 6-hydroxydopamine toxicity [291]. In these studies, CP55,940 and HU-210 agonists exhibited antioxidant properties not mediated by cannabinoid receptors [23]. This antioxidant feature might protect nigro-striatal neurons from death in Parkinson's disease. Furthermore, it was observed that chronic treatment with WIN55,212-2 prevented nigro-striatal neurons from neurotoxicity/neuro-inflammation caused by 1-methyl-4-phenly-1,2,3,6-tetrahydropyridine (MPTP) and ameliorated motor deficit associated with Parkinson's disease [294].

A more recent study showed a protective action of Δ^9-THC in SH-SY5Y neuroblastoma cell line exposed to three relevant toxins, paraquat, lactacystin and 1-methyl-4-phenylpyridinium (MPP+). In the same study, neuronal injury caused up-regulation of the CB1 receptor, however, Δ^9-THC neuronal protective effect was suggested to be mediated by PPAR-γ stimulation [295].

In addition, in an animal model of Parkinson's disease, PEA treatment decreased microglial cell activation. Additionally, chronic treatment with PEA counteracted MPTP-induced motor deficits *via* a mechanism partially dependent on PPARα. These data suggest protective effects of PEA in MPTP-induced neurotoxicity [296]. Taken together, these studies suggest that the endocannabinoid system plays a role in Parkinson's disease, and may be a target for new therapeutic approaches.

HUNTINGTON'S DISEASE

Huntington's disease is an autosomal dominant progressive neurodegenerative pathology that affects 5–10 for every 100,000 people worldwide and causes death within 20 years [297]. Motor disturbances such as chorea, psychiatric symptoms, dystonia and dementia are the main features of the disease [298]. Huntington's disease is caused by unusual increment of huntingtin gene that gives rise at its NH2 terminal domain to an elongated glutamine repeat, that is a trinucleotide polyglutamine (cytosine, adenine, guanine) [299]. The result of this mutation is an altered intracellular toxic protein with abnormal conformations, typically β-sheet structures, resistant to normal cell processes of protein degradation and metabolic pathways [297], indeed, there is ''aggregation'' or ''inclusion'' of this protein within neurons in brain regions of Huntington's disease patients. The exact function of huntingtin is not completely known but it seems to exert a role in gene transcription and in vesicular transport [300, 301]. This disease is characterized by cerebral cortex atrophy, drastic striatal neuronal loss and consistent reduction of GABAergic medium spiny projection neurons [302, 303]. The pathological processes involved in Huntington's disease include the loss of trophic factors, particularly BDNF, excitotoxicity, oxidative stress and inflammation leading to progressive neuro-degeneration. In animal models of Huntington's disease BDNF was reported to be depleted by approximately 35% [304, 305]. The post mortem analysis of cerebral tissue from patients revealed decreased BDNF mRNA levels [306]. These levels have been correlated to a similar phenotype observed in BDNF partial knock-out mice [304]. Thus, BDNF replacement might be considered a possible therapeutic strategy for Huntington's disease. Furthermore, BDNF has been demonstrated to decrease motor dysfunction, cell loss in animal models and excitotoxicity that emerging reports suggest to be involved in the pathophysiology of Huntington's disease [307, 308]. A defective function of the glutamate transporter, GLT1 has been reported in Huntington's disease patients and glutamate uptake was reduced [309]. The subsequent accumulation of extracellular glutamate might be responsible for the enhanced excitotoxicity and the excessive activity of glutamate receptor, N-Methyl-D-aspartic acid (NMDA). Several studies showed that mutated huntingtin binds to mitochondria, disrupts metabolism and up-regulates pro-apoptotic factors and

p53 [310]. Further studies reported in post-mortem analysis of patients enhanced microglial activation which correlates with neuro-degeneration and the severity of the disease [297].

LEVELS OF CANNABINOID SYSTEM COMPONENTS IN HUNTINGTON'S DISEASE

A correlation between the progression of Huntington's disease and the decrease of the CB1 receptor density has been previously observed in the globus pallidus, putamen and caudate nucleus [195]. In the globus pallidus, the degeneration of GABA/enkephalin efferent terminals induces CB1 receptor loss in the external segment [311], indeed, a major loss of CB1 receptor-immunoreactivity and substance P from the internal segment of the globus pallidus has been reported [312]. Recent studies showed that the down-regulation of the CB1 receptor occurs in specific striatal subpopulation like inter-neurons that express neuropeptide Y/neuronal nitric oxide synthase and spiny neurons [313]. Lots of reports evaluated the constituents of the endocannabinoid system both in post-mortem human tissues [194, 195, 314] and in transgenic animals [190, 192, 196, 315-320]. A loss of CB1 receptor density was detected pre-symptomatically [315] as consequence of mutant huntingtin -associated with altered CB1 receptor gene [321]. In mice, genetic ablation of the CB1 receptors exacerbated Huntington's disease symptoms, whereas symptomatology was reduced by Δ^9-THC, thus suggesting that altered CB1 receptor activity may be a main feature of Huntington's disease [321]. On the other hand, in the striatal microglia of the same mice, an increase of CB2 receptor was also observed. Indeed, genetic ablation of CB2 receptors in transgenic Huntington's disease mice resulted in enhanced activation of microglial cells, exacerbated symptoms and decreased life span [322], thus suggesting that the CB2 receptor may contribute to neuro-protection.

In the striatum decreased expression of 2-AG and anandamide, and in the ventral mesencephalon increased levels of anandamide have been reported in rats with Huntington's disease [192]. Additionally, a reduction of the activity of NAPE-PLD and DAGL ~~that are biosynthetic enzymes of endocannabinoids~~ was observed [323, 324]. In the basal ganglia, a hypofunctional endocannabinoid signaling seems to contribute to the hyperkinesia observed in this disease. In the cortex, decreased levels of 2-AG were accompanied by enhanced levels of anandamide and FAAH, while the hydrolytic enzyme MAGL was decreased [323, 324]. Analyzing lymphocyte preparations from patients, it has been observed that anandamide levels of expression were six-fold higher than those observed in control subjects; this might be ascribed to the inhibition of FAAH function in the metabolism of anandamide [325]. However, the activation of the CB1 receptor as therapeutic target in Huntington's disease still remains to be assessed. Contrasting results in rodent models were not able to establish if CB1 receptor agonism has a neuro-protective action, alleviates symptoms, or exacerbates the disease.

Moreover, selective agonists of CB2 receptor reduced neuronal loss suppressing glial activation [322, 326]. An interesting therapeutic option might be represented by growth factor stimulation of endogenous neurogenesis. Overall, these findings suggest the impairment of multiple constituents of the endocannabinoid system in Huntington's disease progression.

THERAPY OF HUNTINGTON'S DISEASE

Actually, therapies to treat Huntington's disease adopt available anti-dopaminergic agents but are limited to reduce symptoms ~~of the disease, which so far cannot be cured~~. Some studies suggest the involvement of the endocannabinoid system in the pathogenesis of this disease, indeed, cannabinoid agonists eliciting anti-hyperkinetic and neuro-protective effects might represent an interesting therapeutic option [327]. Another study described in the sub-ependymal layer of healthy and Huntington's post mortem human brains a new subset of progenitor cells expressing CB1 receptor. This data suggest that these cells might replace cell lost due to neurodegenerative processes [328]. In addition, studies performed in animal models showed that CB1 receptor agonists and endocannabinoid transport inhibitors could decrease hyperkinesia [316, 329]. Of interest, CP55,940, a CB1 receptor agonist exhibited lower effect with respect to AM404, an anandamide transport inhibitor, that presents affinity for the TRPV1 receptor [330]. The ability of AM404 to decrease hyperkinesia [317, 329] might involve the TRPV1 receptor, since VDM11 and AM374, other transport inhibitors that do not bind to TRPV1 receptors were devoid of anti-hyperkinetic properties in rats with Huntington's disease [329], indeed, UCM707, the most potent transport inhibitor known, showed low effect [292, 331, 332]. Notably, the endocannabinoid-vanilloid compound, arvanil alleviated hyperkinesia in rats with Huntington's disease [333]. These findings suggest that TRPV1 receptors or their combination with CB1 receptors might be new therapeutic targets in Huntington's disease [329]. More recently, a Sativex -like combination of phytocannabinoids was used in animal models of Huntington's disease, in which cannabinoid agonists that compose Sativex, Δ^9-THC and cannabidiol used alone, have demonstrated efficacy. In particular, preclinical data support beneficial effect of Sativex as a neuro-protective drug able to delay signs of disease progression in a pro-inflammatory model of Huntington's disease. Furthermore, these results show that both CB1 and CB2 receptors seem to be implicated in the effects exhibited by Sativex-like combination of phytocannabinoids, so reinforcing the broad-spectrum effects of Sativex that combines activity at both CB1 and/or CB2 receptors [334].

Few human trials have been performed to assess the effects of cannabinoid agonists in Huntington's disease, and the data do not seem to be promising as those on animals. Small trials using nabilone, a synthetic THC analog and cannabidiol demonstrated no efficiency or even enhanced choreic movements in Huntington's disease patients [335, 336]. These negative data might be due to dose issue, to lack of TRPV1 receptor activity of the drugs, or to the advanced phase of the disease. Nonetheless, further works are required to investigate cannabinoid therapeutic potential in Huntington's disease.

ALZHEIMER'S DISEASE

Alzheimer's disease is a debilitating disorder accompanied by neurodegeneration that affects more than 26 million

people worldwide [337]. In particular, it is believed that among people over 65 year of age 10% will develop the disease and among people over 80 years of age 25% will develop the disease. These numbers will rise to 1 in 85 persons within the next thirty years [337, 338]. Alzheimer's disease is determined by the progressive impairment of cognitive and memory functions [339]. From an etiological point of view, it is caused by both genetic and idiopathic factors that determine atrophy of neurons projecting to hippocampus and cerebral cortex [340]. Common features are gliosis, neurofibrillary tangles enriched in hyper-phosphorylated tau protein, formation of neuritic plaque enriched in β-amyloid peptide, neuro-inflammation accompanied by cognitive decline favored by astrocytes and microglia [341]. Neuro-inflammatory processes are involved in the pathogenesis of Alzheimer's disease, post mortem evaluation of brains of patients showed increased number of activated astrocytes and microglial cells and significant high production of pro-inflammatory cytokines, TNF-α, IL-6, IL-1, and reactive oxygen species (ROS) [342, 343]. Indeed, clinical evidence suggested a correlation between TNF-α levels and cognitive decline, in fact anti-inflammatory agents have been demonstrated to be able to retard disease onset and progression. Immune cells can recognize fibrillated β-amyloid and the peptide can be phagocytosed. However, when peptides form neuritic plaques by oligomerization and aggregation, the recognition does not occur, and this leads to immune cell chronic activation [344, 345]. Multiple findings suggest the patho-physiological relevance of neuro-inflammation in neurodegeneration in Alzheimer's disease. In addition, further features of Alzheimer's disease are represented by dysregulated intracellular Ca^{2+} and NMDA receptor activation [346]. Excessive NMDA activity and excitotoxicity are consequences of both NMDA receptor activation and glutamate accumulation as a result of β-amyloid -mediated decrease in astrocytic uptake [346, 347]. β-amyloid has been found to enhance voltage-dependent Ca^{2+} channel activity [348] and to form Ca^{2+} permeable pores in membrane bilayers [349]. β-amyloid-mediated excitotoxicity has been associated with the neurodegeneration as rises in intracellular Ca^{2+} have been found to stimulate apoptosis and activate lysosomal cathepsins, calpain and caspase-3 [350, 351]. Activated microglia found in neuritic plaques represent the main source of ROS and oxidative stress in the central nervous system, since ROS can then sustain the inflammatory process by activation of pro-inflammatory pathways [352].

LEVELS OF CANNABINOID SYSTEM COMPONENTS IN ALZHEIMER'S DISEASE

Several studies have been performed to establish if a potential modulation of the components of the endocannabinoid system occurs in Alzheimer's disease. Analyses of post-mortem brain tissues of patients, showed a significant increase of CB2 receptor and FAAH in areas of neuritic plaques like entorhinal cortex and para-hippocampus surrounded by microglia [60, 353]. This increase in CB2 receptor expression might be due to an attempt to counteract chronic inflammation in Alzheimer's disease, since the activation of this receptor reduces both cytokine secretion and microglial cells activation [354]. In a recent study, the expression of the CB1 receptor in the prefrontal cortex of patients with Alzheimer's disease was showed to initially rise at the first stage of disease progression, and then steady decline [355]. Furthermore, CB1 receptor level and G protein coupling significantly decreased in Alzheimer's disease brains, and nitration of both the CB1 and CB2 receptor was enhanced [354]. Lipidomic analyses were performed on post-mortem brains from patients to assess the levels of endocannabinoids. In the midfrontal and temporal cortex reduced levels of anandamide and its precursors were detected [356]. In addition, on plaque-associated astrocytes up-regulation of the metabolizing enzyme FAAH has been observed and, probably as consequence, an increased degradation of anandamide occurred [60]. In a very recent study, plasma levels of endocannabinoids were measured in Alzheimer's disease patients. PEA and 2-AG levels were higher in Alzheimer's disease patients compared to healthy subjects and 2-AG levels were positively related to memory and attention performances, thus suggesting that the increased levels of 2-AG and PEA with ongoing pathological processes might modulate cognitive performances [357].

THERAPY OF ALZHEIMER'S DISEASE

Considering the lack of efficient therapies to cure Alzheimer's disease, one of the main objectives is to find effective treatments. Emerging findings suggest that targeting the endocannabinoid system might be of therapeutic interest since it is a powerful modulator of neuronal activity and inflammatory processes [358, 359].

In rat microglial cells, CB1 receptor activation dose dependently blocked nitric oxide production, involved in the neurotoxic effects of β-amyloid peptide [43]. In cellular models of Alzheimer's disease, anandamide prevented neurotoxicity induced by β-amyloid via CB1 receptor-mediated stimulation of the ~~mitogen-activated protein~~ kinase signaling [360]. In PC12 cell line~~s~~, cannabidiol elicited protection against β-amyloid-induced neurotoxicity [361]. Notably, CB1 receptor inhibition by the antagonist SR141716 ameliorated in mice the cognitive deficit induced by β-amyloid peptide, probably by enhancing hippocampal acetylcholine [362]. In rats, WIN 55,212-2 prevented microglial activation induced by β-amyloid, cognitive deficit and loss of neuronal markers. Furthermore, JWH-133, WIN 55,212-2 and HU-210, inhibited microglia activation induced by β-amyloid, as determined by cell morphology and mitochondrial activity; these effects were not dependent on the antioxidant action of ligands. In addition, cannabinoids were able to abrogate neurotoxicity mediated by microglia after β-amyloid addition to cortical co-cultures in rats [354]. A very recent study showed that in AβPP/PS1 transgenic mice THC or cannabidiol botanical extracts or their combination administered in the early symptomatic phase, preserved memory of these mice. A significant reduction of Aβ42 peptide levels and changes in the composition of plaques were detected in mice treated with a combination of THC and cannabidiol, suggesting a cannabinoid-induced decrease of the toxic effects of the β-amyloid peptide. Moreover, the combination of THC and cannabidiol reduced learning impairment in these mice. Reduced astrogliosis, microgliosis, and inflammatory-related molecules were also observed, more markedly after the treatment with combination of THC and cannabidiol than with THC or cannabidiol used separately. The redox protein

thioredoxin 2 and the signaling protein Wnt16 were identified as substrates for the effects of THC and cannabidiol. These data demonstrate that the combination of THC and cannabidiol favors a better therapeutic profile with respect to those observed using each *cannabis* constituent alone, thus the use of *cannabis*-based drugs might be used for a potential therapy of Alzheimer's disease [363]. Interestingly, an open-label pilot study tested the effects of dronabinol, on six patients in the late phase of dementia (one patient affected by vascular dementia and five patients affected by Alzheimer's disease): two weeks of treatment significantly ameliorated motor activity and decreased aggression and agitation, without unwanted collateral effects [364].

CONCLUSIONS

In this report, the intriguing role of the endocannabinoid system has been highlighted in main neurodegenerative diseases. In neurological disorders the constitutive elements of the endocannabinoids system can be modulated, for example endocannabinoid release and degradation, and the expression of cannabinoid receptors. Furthermore, the CB1 receptors can release other neurotransmitters and can cross-talk with other neuronal signaling pathways, thus highlighting a complex interplay among numerous factors of these intercommunicating pathways in neurological disorders. In addition, the alteration of elements of the endocannabinoid system in neurodegenerative diseases suggests that pharmacological intervention by use of ligands of cannabinoid receptor, such as agonists or antagonists might be useful to restore a perturbed system that might be the consequence of the progress of a particular disease. We have highlighted in each pathology described, multiple sclerosis, Alzheimer's, Huntington's and Parkinson's diseases how the endocannabinoid levels, the up-regulation of their receptors or modulation of their synthetic or degradative enzymes can be modulated, suggesting that they might represent a target or exert a relevant role in the prediction of disease progression. In addition, several reports indicate that cannabinoid-based drugs that exhibit selectivity for different components of the endocannabinoid system, might provide benefits for example in basal ganglia disorders, like Huntington's and Parkinson's disease ~~but~~, also in demyelinating diseases like multiple sclerosis. These effects include not only the amelioration of motor symptoms but also retard the progression of the disease as consequence of their neuro-protective effects.

CURRENT & FUTURE DEVELOPMENT

Current therapies for neurodegenerative diseases are still not sufficient: indeed the toxic effects of usually used drugs constitute the rule and often long-term side effects are not predictable. We believe that the research ~~aiming~~ to target constituents of the endocannabinoid system or to develop novel *cannabis*-based drugs (without psychotropic effects) should be pursued. This research will help to provide new perspectives of therapy and new agents to be used alone or in combinatory treatment to open the way to better pharmacological approaches of intervention.

ABBREVIATIONS

Δ~~9~~-THC	=	Δ~~9~~-tetrahydrocannabinol
TRPV1	=	Transient receptor potential vanilloid ~~receptor~~ type 1
PKA	=	Protein kinase A
PKB	=	Protein kinase B
CHO	=	Chinese hamster ovary
PPAR	=	Peroxisome proliferator-activated receptors
~~NAPE-PLD~~	~~=~~	~~NAPE-selective phospholipase D~~
~~FAAH~~	~~=~~	~~Fatty acid amide hydrolase~~
~~DAGL~~	~~=~~	~~Diacylglycerol lipases~~
~~MAGL~~	~~=~~	~~Monoacylglycerol lipase~~
BDNF	=	Brain-derived neurotrophic factor
A1Rs	=	Adenosine A1 receptors
~~RR-MS~~	~~=~~	~~Relapsing – remitting multiple sclerosis~~
~~PP-MS~~	~~=~~	~~Primary progressive multiple sclerosis~~
PEA	=	Palmitoylethanolamide
CREAE	=	Chronic relapsing experimental autoimmune encephalomyelitis
CAMS	=	Cannabinoids in Multiple Sclerosis
NMDA	=	N-Methyl-D-aspartic acid

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interests.

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